

MEMBRANE TECHNIQUES USED FOR THE ASSESSMENT OF THE MICROBIOLOGICAL SAFETY AND QUALITY OF THE FOOD PRODUCTS

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Abstract

In the case of food industry it is appropriate to set microbiological criteria defining the acceptability of the processes, and also food safety microbiological criteria setting a limit above which a foodstuff should be considered unacceptably contaminated with the micro-organisms for which the criteria are set. Membrane filtration techniques are mainly used to analyze from the microbiological point of view, the food drinks, here including water, wine, beer, soft drinks, and mineral water. The nutritive disks are always used associated with membrane filters and membrane techniques. These are designated especially to perform specific analysis for one particular micro-organism or a group of micro-organisms or for a specific substrate such as water, wine, beer. The present paper presents the results for tests performed in order to assess the microbiological safety and quality of several Romanian food drinks – mineral water, wine and beer.

Keywords: *food microbiology, food liquids, membrane filtration, food safety, microbiological analysis.*

Introduction

Although the Romanian sanitary legislation is almost entirely harmonized with the EU legislation, the conditions for producing, storing, transport and marketing of food are regulated and the responsibility for food producers and distributors, the organization of official control and sanctions/punishment in respect to the food quality protection are settled. It is very important for all food business operators to reconsider their attitude against food safety under circumstances of food industry in Romania trends to meet all requirements of the modern food production (Begea, 2006).

Membrane Techniques Used for the Assessment of the Microbiological Safety and Quality of the Food Products

Up to the end of 2005 there were numerous pieces of legislation covering the hygiene of specific commodities and many of these included microbiological criteria. From 1 January 2006 a new package of food hygiene rules has replaced these pieces of legislation. The existing microbiological criteria were reviewed to produce a new Regulation (Regulation 2073/2005) which supports that package. Many of the existing criteria remain unchanged, but some no longer exist and criteria have been established in new areas where this is necessary. The Regulation and associated national legislation applies to all food business operators involved in the processing, manufacturing, handling and distribution of food, including retailers and caterers (General Guidance, 2005).

Microbiologists consider that at present there are no general methods to accomplish the particular requirements for the specific analyse of various food products. Only the culturable methods ensure the conditions for the quantitative and qualitative assessment and for the microbiological characterisation of micro-organisms. These assessments evaluate the microbiological contamination degree through the determination of the colony-forming units (*cfu*), the exams being correlated with qualitative studies with contamination micro-organisms isolation and characterisation (Bahrim, 2003).

The microbiological control comprises several steps, such as the test of sterility, detection, isolation, enumeration and identification of micro-organisms and their metabolites in different materials and products.

The most important characteristic of a microbiological analyse is the accuracy. Using the membrane filtration technique the question is how similar the results for these tests in comparison with classical standard methods are and also what is the variability associated with this technique.

The precision and the specificity of tests are very important because the false negative results could lead to recalls of the food products from the market and could bring about financial damages for the producing companies and consumers satisfaction. False positive results could lead to the break or delay in food products distribution on the market.

Membrane filtration techniques are mainly used to analyze from the microbiological point of view, the food drinks, here including water, wine, beer, soft drinks, and mineral water.

The nutritive disks are always used associated with membrane filters and membrane techniques. These are designated especially to perform specific analysis for one particular micro-organism or a group of micro-organisms or for a specific substrate such as water, wine, beer.

Experimental

We should mention that the fast modern culturable methods does not have in all cases as cause the reducing of the incubation time in comparison with classical methods, but is the effect of the simplifying of the work methodologies. Thus through direct inoculation the steps of sampling and sample preparing are eliminated and using specific culture media the selective developing of studied micro-organisms is achieved (Bahrim, 2003).

Microbial contamination of water, food, beverages needs to be monitored for the following main reasons:

- to maintain compliance with National / International regulatory requirement & Internal company guidelines;
- to provide the most important means of determining if the process is performing correctly & process optimization.

Advantages and limitation for membrane filtration method in comparison with direct inoculation method are presented in table 1.

The microbial monitoring using membrane filtration technique can be performed for the next types of beverages: Non-alcoholic Beverages (Soft Drinks), with two subclasses (Carbonated and noncarbonated); Juice; Sugar water; Ready to drink teas; Sport drinks; Bottled water; Alcoholic Beverages; Wine; Beer; Miscellaneous (mead, cider).

Validation is essential for selection of best membrane, medium and incubation conditions for optimal results.

Steps of microbiological monitoring using membrane filtration technique are the following: collect samples, analyse, culture, incubation, enumerate and identify (ISO 8199).

Table 1. Membrane filtration versus Direct inoculation

Membrane filtration		Direct inoculation	
Advantages	Limitations	Advantages	Limitations
Rinse away inhibitory or preservatives agents	Non filterable samples	Fast, Easy	Limited sample size: lower sensitivity
Sample concentration <ul style="list-style-type: none"> ▪ high volume sample can be filtered, not aliquot ▪ Statistically more valid ▪ More sensitive: 1 CFU/volume 	High solids may plug membrane	<ul style="list-style-type: none"> ▪ Wide selection of media ▪ Non-filterable samples 	Small Colonies

Membrane Filtration system used in order to apply the membrane filtration technique has the next components / characteristics:

- the sterile filtration apparatus, that should be connected to a source of vacuum, to draw a sample through a membrane filter;
- the organisms are collected on the membrane filter:
 - ❖ 0.45 µm filter (for the best retention of bacteria; for the best recovery)
 - ❖ 0.8 µm filter (for a complete retention of yeast & mould; for a improved throughput (but it is hard to filter beverages);
- Can filter any volume range (< 10 ml: dispensed into 30 ml of sterile diluents).

The producers and currently recommends 0.45 µm membranes because 0.45 µm provides better flow and consistent recovery of a broad range of micro-organisms.

For our experiments the next types of membranes (Dr. Moeller & Schmelz, Germany) were tested: Wine-ND – for the harmful micro-organisms for wine; Wort-ND – for yeasts & moulds; Sabouraud-ND – for yeasts & moulds; Plate-Count-ND – for TPC; MacConkey-ND –

for *E. coli*, Coliforms; Orange serum-ND – for the harmful micro-organisms for beverages.

A measured volume of liquid sample, or addition of the sample, is filtered through a membrane filter that has filtration characteristics equivalent to a reted pore diameter of 0.45 µm. The membrane filter is placed on the selective medium and incubated under the conditions specified for the medium. Place each membrane on a Petri dish containing the specific medium, ensuring no air is trapped beneath. Plates containing membrane on absorbent pads should always be placed in an air- or water-tight container to prevent desiccation of the medium.

With membranes of mean pores diameter 0.45 µm, it may be possible to filter several liters of such water through a single membrane, and so achieve a high level of test sensitivity.

A test volume of the sample or a dilution of it should be chosen to yield less than about 100 colonies on a membrane of 47 mm or 50 mm in diameter (ISO 8199).

The volume of sample is determined by the bacteriological density and is depends on the type of food liquid analysed. The maximum sample volume is recommended in order to result 20 – 200 *cfu* per filtration membrane.

Generally speaking, the sample volume is 100 ml. For the very contaminated samples a smaller sample volume is analysed. Because it is extremely difficult to analyse small volume of sample with a high accuracy, several dilutions are required to be analysed.

The *cfu* value is obtained divided the number of colonies by the sample volume of 100 ml. The next equation is applied to calculate the *cfu* for a single membrane:

$$cfu = \frac{\text{No. colonies enumerated}}{\text{ml filtered sample}} \times 100$$

The syntagma “*ml of filtered sample*” refers to the volume of sample and not to the volume of dilution. If the *cfu* is higher than 200 for a membrane or if the colonies are hardly to be detected (to be identified and enumerated) the analysis should be repeated and the dilution will be chosen in a such way to obtain ca. 50 colonies and less than 200 colonies.

Results and Discussion

Taking into account the national legislation, the microbiological criteria for the main food liquids are those presented in table 2. In the table 3 the parameters for which methods of analysis are specified.

Table 2. Microbiological criteria for food drinks (Romanian legislation)

Food drink	Microbiological criteria requested by the national legislation
Drinking water (Law 458/2002, completed with Law 311/2004)	Colony count 22°C Colony count 37°C <i>Enterococci</i> <i>Pseudomonas aeruginosa</i> Coliform bacteria <i>E. coli</i> <i>Clostridium perfringens</i>
Mineral water (HG 1020/2005)	Coliform bacteria and <i>E.coli</i> <i>Enterococci</i> Sulphite-reducing anaerobic bacteria Colony count 22°C Colony count 37°C
Beer (OMS 975/1998)	Coliform bacteria <i>E. coli</i> Yeasts and moulds
Wine (OMS 975/1998)	Yeasts and moulds
Soft drinks (OMS 975/1998)	Total plate count Coliform bacteria <i>E. coli</i> Yeasts and moulds

Table 3. Parameters for which methods of analysis are specified

Coliform bacteria	ISO 9308-1 / STAS 3001/91
<i>E. coli</i>	ISO 9308-1
<i>Enterococci</i> (faecal streptococcus)	ISO 7899-2 / STAS 3001/91
Colony count 22°C	EN ISO 6222 / STAS 3001/91
Colony count 37°C	EN ISO 6222 / STAS 3001/91
<i>Pseudomonas aeruginosa</i>	EN ISO 12780 / STAS 3001/91
<i>Clostridium perfringens</i>	SR ISO 6461-1;2/98 STAS 3001/91

As regard the influence of pore size on micro-organism recovery, two aspects have to be considered:

- *Culturability*: the larger pore sizes allow colonies to grow bigger (maybe because of the easier access to media)
- *Retention*: the larger pore sizes tested (1.2 μm and 0.8 μm), allowed significant passage of a small organism, but not the 0.45 μm or 0.22 μm pore size.

The next aspects have also to be taken into consideration:

- Each microorganism has the potential to react differently.
- The standard 0.45 μm filter is the most appropriate filter for general microbiological purposes
- Larger pore sizes offer higher flow rate (and throughput)
- Optimum recovery is a balance between retention and culturability.

Abbreviations used: *TPC* – total plate count; *cfu* – colony-forming units

Conclusions

The present paper had as aim to emphasize the microbiological criteria for food liquids focusing on the membrane filtration techniques as one of the modern microbiological method. Every testing laboratory for foodstuffs should be deeply involved in ensuring with high accuracy and in time of the test results for physical-chemical and microbiological analysis, in order to produce safe and nutritive food products. These membrane filtration techniques can be the basis for the adequate monitoring programs within the Romanian food factories.

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